UNIVERSITY OF CANTERBURY

Genetic parameter estimates for growth traits of *Eucalyptus bosistoana*

Assessment of two progeny trials in Marlborough, New Zealand

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1.0 Abstract

This paper presents a follow up study to that was already completed by Luis et al. (2011). Luis et al (2011) investigated the survival of *E. bosistoana* half-sib families within two progeny trials in Marlborough, New Zealand. The study investigated genetic growth parameters of the same two progeny trials. Trees at Craven (progeny trial) were statistically taller and larger in diameter than Lawson (other progeny trial), which is likely to be a result of thinning occurring one year earlier at the Craven and Lawson North sites than at the Lawson East site.

The progeny trials were set up into randomized incomplete blocks which allowed effective calculations of fixed and random effects from a mixed-effect linear model. The family, incomplete block and residual variances from the mixed-effect model made heritabilities of growth traits possible to calculate. Narrow sense heritabilities for diameter at breast height ranged from 0.13 to 0.18, while tree height heritabilities ranged from 0.1 to 0.17.

The inter-site correlation of family performance was weak to moderate for both height;

- Craven : Lawson North = 0.28
- Craven : Lawson East = 0.44
- Lawson East : Lawson North = 0.27

And diameter at breast height (dbh);

- Craven : Lawson North = 0.32
- Craven : Lawson East = 0.39
- Lawson East : Lawson North = 0.36

Heritabilities were weak to moderate for growth traits but there was substantial variation so selection is still likely to provide sufficient gain. Further studies will need to consider researching the genetic parameters of wood qualities of *E. bosistoana.*

Eucalyptus bosistoana, quantitative genetics, breeding, progeny, heritability

2.0 Table of Contents

3.0 Acknowledgements

- I would like to thank my supervisor Luis, although our chats were sometimes infrequent, your help and advice was invaluable.
- NZDFI and Bruce Manley for the opportunity to work with such a great outfit and allowing my summer project to evolve into an honours project.
- My fellow students who sympathised the tough times but were great bunch to have had the chance to work with.

4.0 Introduction

New Zealand has just less than 1.8 million hectares of plantation forest, which equates to roughly 22% of total forest area in New Zealand. The major species is *Pinus radiata* which comprises 90% of plantations in new Zealand, eucalypt trees have only ever been of minor importance to the New Zealand Forest Industry where knowledgeable persons would plant them based on the guarantee that they knew they could sell them (Wilcox, 1980). Comparing New Zealand's plantation estate to other countries, other countries have more often than not 50/50 splits with two types of forest plantations. So why has New Zealand had all of its eggs in one basket since *Pinus radiata* was first brought here in the 1930s.

Pinus radiata is not naturally durable; it is a softwood species which is often treated with Copper-Chrome-Arsenate (CCA) to maintain its structural and biological integrity. CCA has been used world-wide for approximately 60 years and since the 1950s in New Zealand. The problem is that the timber cannot be disposed of environmentally safely. The use of naturally durable eucalypts as an alternative to CCA treated wood could facilitate markets, such as; post and poles, lamp posts, power pole cross arms, or railway sleepers (Hedley, 1997).

NZDFI are a research outfit based in Marlborough, New Zealand. Their aim since its inception in 2003 has been to promote the use of alternative naturally durable timbers over treated pine. They have been planting progeny and species trials since 2003 but picked up a lot of funding just before 2009, which allowed them to establish any more trials. Some of the species they are interested in breeding are *E. bosistoana, E. quadrangulata, E. globoidea, E. camaldulensis, and E. argophloia* to name a few. *E. bosistoana* is a relatively unknown timber as not much research has been completed on it but it has been rated by an Australian standard as extremely naturally durable (Bush, 2011). It is hoped that NZDFI's breeding programme will provide genetic material that can be deployed on a commercial scale in the years to come. For right now, the focus is on research as much as possible while these progeny and species trials are growing.

For a breeding programme to be successful, it is imperative that for the trait of interest, extensive variation exists within the population and that when selections are made, the progeny of selected trees display effective inheritance of the trait of

interest i.e. trait is heritable. According to Apiolaza et al. (2011), the first priority when introducing a new species into a new environment is to select for growth rate, adaptation, and health. Luis' study completed an assessment on early growth and survival so the purpose of this report was to add to the knowledge around growth parameters of *E. bosistoana* and the whether the variation being observed is being caused by additive genetic sources or environmental conditions.

5.0 Problem Statement and Objective

- Is the degree of genetic control on growth traits consistent with current and past literature?
    - Is the degree of genetic control on diameter = 0.2?
    - Is the degree of genetic control on tree height = 0.25?
- Is inter-site family correlation consistent with current and past literature?
    - Is inter-site family correlation = 0.7?
- Are there any spatial or operation explanations for some of the growth trait variation?

The objective of this report is to add information and knowledge to an area where little is known. *E. bosistoana* has not been researched thoroughly and it only exists as natural, primary forest outside of New Zealand thus, it has not been influenced by breeding objectives. It is my hope that these results can be used and consulted with in the process of making selections for plus trees from the progeny trials within this report.

6.0 Review of Literature

6.1 Forest tree breeding, past and present

The earliest domestication of plants and animals began approximately 10,000 years ago during the Mesolithic period. Among the first domesticated plants were; potatoes, peas, lentils, beans, wheat. Slash and burn was the technique used to provide the suitable land areas to grow crops leading to severe deforestation, which continued until roughly 3,000 years ago when civilisations developed ideas pertaining to the silviculture of forest systems (coppicing, plantation establishment) and saving the seed from the phenotypically superior agriculture plants (Sands, 2005); (White, Adams, & Neale, 2007).

It wasn't until 1856 when Louis de Vilmorin developed progeny testing to rank individual seed trees (Briggs & Knowles, 1967) and in 1866 when Gregor Mendel studied inbred lines of peas and developed the laws of diploid inheritance. Years later, in 1908, Godfrey Hardy and Wilhelm Weinberg established a relationship between allele and genotype frequencies which is the foundation of population genetics. The statistician Sir Ronald Fisher set the standards for the design, analysis, and randomization for experimental methods, which are still used today (White, Adams, & Neale, 2007).

Increasing world population throughout time has put increasing pressure on the ability of natural resources to supply societies with minerals, food, commodities, and water. This has led to more awareness around the importance of conserving genetic material (a relatively benign species now may be significantly important in the future) (White, Adams, & Neale, 2007). It has also meant that breeders are faced with challenging tasks; breeding trees for complex environments (often bordering agriculture land), breeding for many objectives but no certainty around the future management strategy, and improving traits of tree species that have a mixture of genes affecting single or multiple traits.

Since the mid-1950s, simple selection-type breeding principles have identified that gains in yield (volume per hectare) can be increased and undesirable traits can be modified. Some forest management practices have used high intensity selections within breeding programmes but did not consider constructing efficient breeding operations for multiple possible environments (matching genotype to site) or for;

conflicting traits and complex gene actions (Namkoog, Kang, & Brouard, 1988). Reviews of the research completed in the early chapters of tree breeding were directed at the variation in natural stands, small trials or provenance tests of species, trying to explain geographical variation of species and morphological differences (Eldridge, Davidson, Harwood, & van Wyk, 1993).

More recently, eucalypt breeding programmes (globally speaking) have advanced considerably with international conferences held in a number of locations where breeding and genetic improvements of eucalypts are the major theme. *E. grandis* is an example of a species being developed within advanced breeding programmes in South Africa, Brazil, and the USA, whereas *E. globulus* and *E. nitens* are more common species of choice in breeding programmes in other parts of the globe (Volker, 2002). Studies and recording of quantitative genetics (variance and heritabilities) of eucalypts did not begin until the 1970s and were not extensively carried out until the 1990s. Despite this, breeding and selection programmes proceeded via seed production stands and open pollinated seed orchards thus, were simply programmes exploiting genetics of base populations dependent on accuracy of genetic parameters within the progeny (Eldridge et al. 1993).

With regard to tree breeding in New Zealand, *Pinus radiata (*D.Donn) has been the predominant focus since the 1950s. Other than *Pinus radiata,* New Zealand has bred other coniferous species but in terms of *Eucalyptus spp, E.nitens* has been the only one to receive attempts at improving it (Volker, 2002). Initially phenotypically outstanding trees (plus trees) were selected and in turn had seed and grafts collected from. It was quickly realised that breeding was going to be an ongoing process; gains would be cumulative over multiple generations rather than being realised from the first selections and progeny trials (Burdon, 2008).

Fifteen years after the first efforts (Burdon, 1992) to breed *Pinus radiata* (D.Donn) in New Zealand, tests began to focus on a wider genetic base (material from complete geographical range of species, and many more plus trees selected), which led to a hierarchy of genetic material dispersed across production populations (intensive genetic improvement, low variability) and breeding populations (less improvement but broad genetic base). From this point, breeding goals evolved from vigour and form to wood properties (Burdon, 2008). This is yet another example of how difficult it is to conduct breeding programmes when the purpose of objectives changes. There are two necessary conditions for success in breeding;

variation in the trait of interest and the effectiveness of choosing plus trees (Namkoog, Kang, & Brouard, 1988).

6.2 Eucalyptus bosistoana – What, where, why?

E. bosistoana belongs to a large section within the subgenus *Symphyomyrtus* (the boxes and ironbarks), section *Adnataria.* Species placed in the *Adnataria section* can be found in all mainland States of Australia though; the major distribution is in Queensland and New South Wales with smaller numbers present in South Australia and Victoria. Most species have rough bark. Juvenile leaves are petiolate, anthers are adnate, ovules are in four vertical rows and the seeds are reticulate on the dorsal side. *Adnataria* species differentiate themselves from other *Symphyomyrtus spp.* in that their vegetative bud at the growing tip of a group of inflorescences on leafless branchlets usually aborts, leaving a terminal inflorescence The name Symphyomyrtus was originally given as a generic name to eucalyptus lehmannii from coastal southern Western Australia, and refers to the joined fruit clusters (from the Greek *symphyo*, joined, and *mytos*, a myrtle). It is the largest subgenus within the Eucalypts and consists of several hundred species (Boland, Brooker, & McDonald, 2006).

E. bosistoana is the largest of the box group of eucalypts; it commonly reaches 30 to 36 metres in height and from 60 to 100 centimetres in diameter at breast height, although greater sizes have been observed. The trunk is usually of good form and more than one-half of the total height, whilst the crown is relatively small and compact for a box. It mainly grows on coastal areas of eastern Victoria and the coast of New South Wales, south of Sydney, though it is nowhere very common. The most important occurrence is in the Cann River area of Victoria. Generally a lowland coastal species growing near rivers and streams, *E. bosistoana* does occur on the lower slopes of the southern tablelands of New South Wales (Boland, Brooker, & McDonald, 2006).

The altitudinal range of the main occurrence is from just above sea level to about 150 metres, but in the northern limits of the range the altitude extends to over 300 metres. *E. bosistoana's* natural climate is mainly cool temperate, with mild to warm summers and cool winters. The annual rainfall across its range is within the limits

of 635 and 1000 millimetres, and precipitation is distributed fairly uniformly throughout the year (Forestry and Timber Bureau, 1962). *E. bosistoana* shows a marked preference for better quality soils, especially good loams over limestone. It will grow both on soils periodically waterlogged and on somewhat dry sites. Unlike most other boxes, which are typically species of savannah woodland formation, this is a forest species.

Bosistoana's wood is light brown, sometimes with a pink tinge or pink streaks, uniform texture, interlocked grain. The density ranges from 1000 to 1200 kilograms per cubic metre and it is considered very strong, very durable and very hard. The timber is only available in limited quantities in localities that have natural stands nearby. Due to its distinctive qualities it is almost entirely used for heavy constructional purposes in the form of sawn beams, piles, poles, railway sleepers and fencing posts (Forestry and Timber Bureau, 1962).

A study on the timber trees of Australia drew conclusions from; scientists, sawmillers, and construction contractors on a variety of timbers in Australia. Witnesses uniformly speak most highly of *E. bosistoana;* stating that care should be taken to preserve this durable timber species, that it had no faults of any kind, and it has been found to be one of the most durable and serviceable of the Eucalypts in Victoria especially in damp conditions (Maiden, 1917). Supporting these early findings, Bush (2011) reported that the natural durability of *E. bosistoana* met class 1 durability ratings in-ground and above ground and was given a class 3 durability rating for marine use (this equates to a life expectancy >25, >40, and 21-40 years in-situ respectively). The ratings were derived from the Australian standard 5604-2005 and refers to the probably life expectancy of heartwood under the three classes of exposure.

6.3 Quantitative genetics

Quantitative genetics is the study and measurement of polygenic traits of trees to estimate genetic control. Phenotypic variation is partitioned into portions caused by genetic and environmental influences (White, Adams, & Neale, 2007) and the degree of genetic control can then be determined. Given that polygenic traits are influenced not only by the gene loci additive effects and non-additive effects, but they are greatly influenced by the environment between sites and within the same

site, so randomized trial design and statistical methods are used to ensure the estimation of genetic control is being calculated as accurately as possible.

For trials in a Randomized Complete Block (RCB) with large numbers of genotypes ('families') large plots are required to accommodate all genotypes to fit within one plot. Larger plots will lead to an unreliable assumption that comparisons between genotypes (in the case of the progeny trials assessed in this report) would be made at the same level of precision regardless of their physical distance. It is therefore more practical to have progeny trials set up in a randomized incomplete block design so genotypes are not placed in the same location within each incomplete block and all genotypes are spread evenly across sites (Williams & Matheson, 1949).

E. bosistoana is a native plant to Australia and therefore has not been planted as a commercial plantation species; instead it has continued to be harvested from the remaining natural stands. There has been limited research that has been completed on this particular species thus. The only estimates of heritability and genetic correlation for *E. bosistoana* that could be found is from a study completed by Apiolaza et al. (2011) in New Zealand as part of New Zealand Dryland Forest Initiative's (NZDFI) broad breeding programme of durable eucalypts. The study reported heritabilities for height at three different sites that ranged between 0.10 and 0.14. Apiolaza et al. (2011) also found that the correlation of family performance between the same sites was on average 0.81.

As a result of the limited research existing for *E. bosistoana,* other papers examining genetic parameters of eucalypt species were sourced (Bush, McCarthy, & Meder, 2011) (Gapare, Gwaze, & Musokonyi, 2003) (Volker, 2002). Furthermore, papers that were not necessarily eucalypt species but the design of the experiments were similar to that of the one being investigated as part of this report (Williams & Matheson, 1949) (Ukrainetz, Kang, Aitken, Stoehr, & Mansfield, 2008) (Steffenrem, Kvaalen, Hoibo, Edvardsen, & Skroppa, 2009). Taking an average of these sources, the narrow sense heritabilities of growth traits that will set the benchmark for this report are 0.2 and 0.25 for diameter at breast height and tree height respectively. Inter-site correlations were not as common in many papers however; most of the above reports provide evidence that an inter-site correlation of 0.7 could be expected.

7.0 Methods

7.1 Study site stand characteristics

Two progeny trials (sites; Lawson and MDC, see figure 1 for map) situated in Marlborough were established during the spring of 2009 by NZDFI. Both sites were initially planted at a stocking rate of approximately 2300 stems per hectare (tree spacing of 2.4 metres between trees and 1.8 metres between rows) with seedlings represented by 66 plus trees. The seed from the 66 plus trees can be divided into 3 groups as they were supplied by 3 different sources;

- Family codes 1 – 20 were supplied by Forest Seeds Australia.
- Family codes 21 – 54 were supplied by Forests New South Wales.
- Family codes 55 – 73 were supplied by Commonwealth Scientific and Industrial Research Organisation (CSIRO).

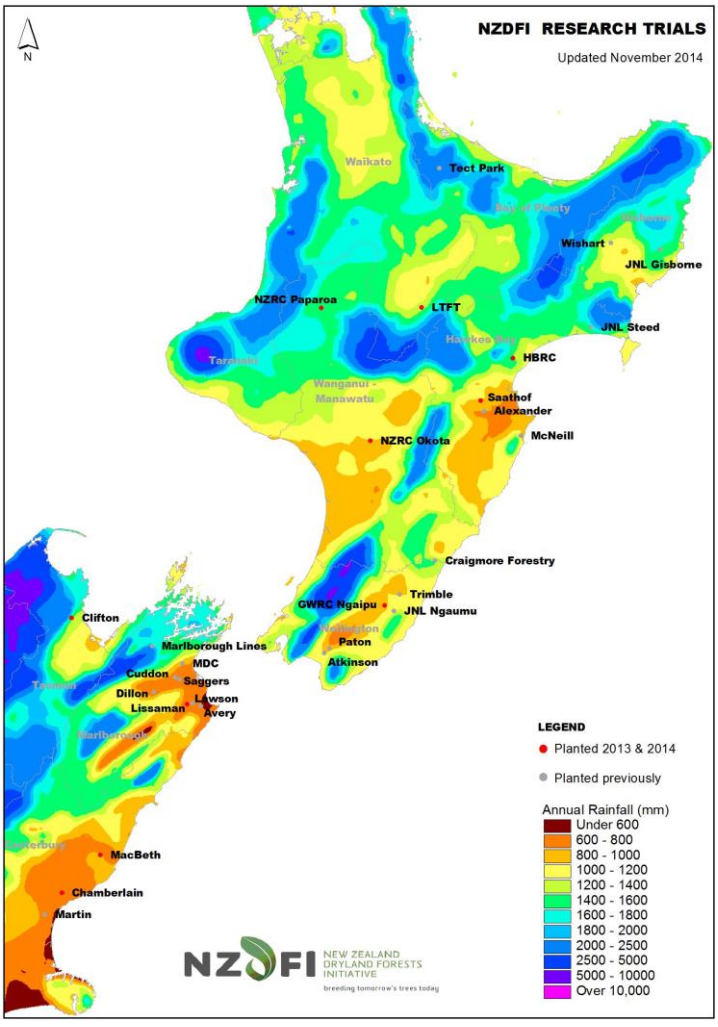


Figure 1: NZDFI research trials of ground durable eucalypt species. Lawson and MDC indicate trials of interest for this project (red arrows)

All sources of seed were derived from the southern coastal localities of *E. bosistoana* (figure 2). As well as the *E. bosistoana* progeny planted at Lawson and Craven, control plantings of *E. globoidea* from 2 localities (Swifts creek and Cann River) and control plantings of *E. quadrangulata* from Cann River.

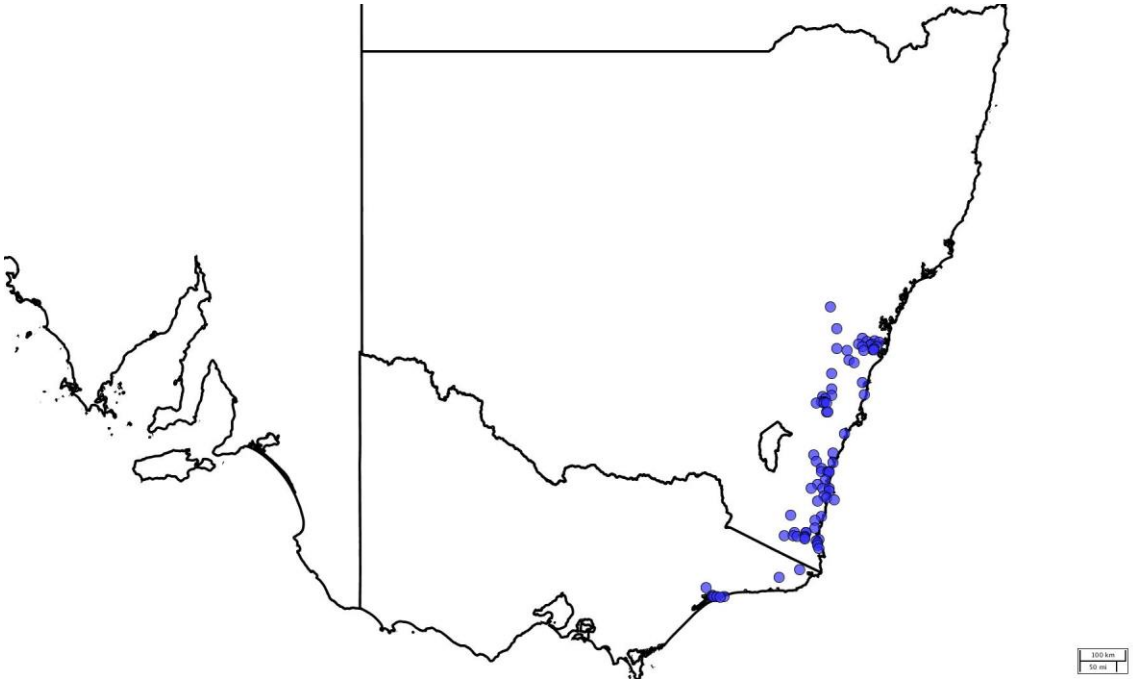


*Figure 2: Distribution of southern and coastal **E. bosistoana**, map retrieved from http://www.ala.org.au/ (Atlas of Living Australia)*

The Lawson site is located approximately 25 kilometres south of Blenheim inland of the small township Seddon (latitude -41° 43' 11.2368": longitude 174° 2' 2.4684"). The Craven site is located approximately 8 kilometres north of Blenheim adjacent to the Wairau River that runs along the foothills of the Marlborough Sounds (latitude -41° 26' 32.4924": longitude 173° 56' 6.7596").

The Lawson site can be divided into two aspects, north and east. One PSP is located on the northern aspect while the remaining three PSPs are located on the eastern aspect. The Lawson has a slope ranging from 16° – 25° and the soil consists of loess on top of massive mudstone whereas the Craven site only has a slight incline (3°) and is comprised of alluvial soils (gravels and fines).

Management decisions were made by Paul Millen (NZDFI Project Manager) thus; access pruning was carried out when the canopies at the trials began to encroach on one another. The whole Craven trial was thinned down to approximately 800 stems per hectare during the 4th growing season (2013/2014). The northern

aspect of the Lawson site was thinned during the same time period; however the eastern aspect at the Lawson site was thinned to a similar stocking as Craven during the 5th growing season.

7.2 Operation description

Measurements of dbh and tree height were electronically recorded from the PSPs at both sites. As well as recording dbh and tree height for all observations in the PSPs, NZDFI asked us to record the form of the trees (broken top, double or multi-leading), tree status (alive, dead, toppled, damaged, or incorrect species), and the flowering status (bud or capsule development, inflorescence present, or mature/empty seed present). For the purpose of this dissertation, I was only interested in the growth traits of *E. bosistoana* so only dbh and tree height were evaluated.

To begin with, Craven had only three PSPs set up while Lawson had four as stated earlier so, Kathryn and I set up one more at Craven before measurements were recorded (PSP 5). This simply involved marking the corner posts of the PSP with blue paint and marking all trees inside the PSP with blue paint also. All other trees within the existing PSPs were also re-marked with blue paint.

For the majority of trees, diameters could be measured 1.4 metres up the stem with a diameter tape. For trees with small diameters (<25 millimetres), a set of callipers were utilised to accurately record dbh, dbh measurements were recorded to the nearest millimetre. Tree height was measured with a height pole specifically designed for completing forest inventory. The pole could be extended to 9.5 metres thus, it was appropriate for the majority of trees. For trees that were above 9 metres, the pole was used in conjunction with a vertex as accuracy while measuring the trees was a priority.

7.3 Study design

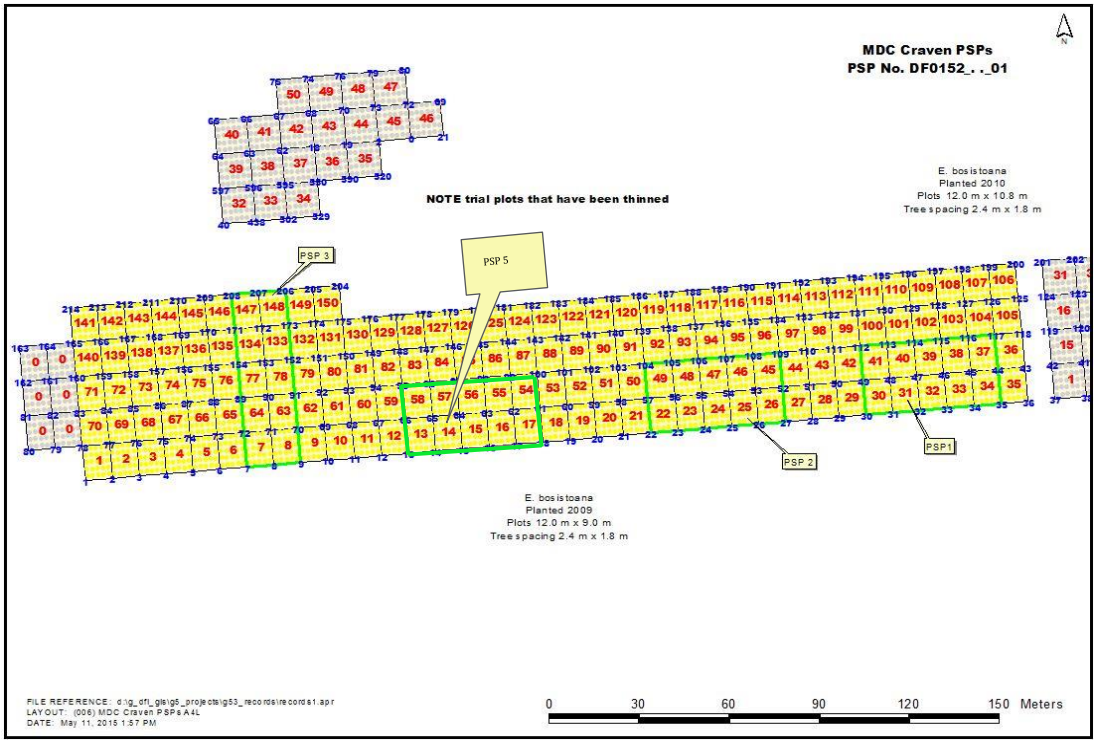
Both the Craven and Lawson trials have a total of 150 incomplete blocks and four PSPs (figures 3 and 4). All Craven PSPs are the same size however, the eastern aspect PSPs at Lawson are slightly smaller than that of the northern aspect PSP. Although the tree spacing at both sites is exactly the same, Lawson incomplete blocks contain 30 tree spaces whereas the Craven incomplete blocks have spaces

for 25 trees. This therefore influences the number of incomplete blocks that comprise a PSP at each site.

Craven PSPs are made up of 10 incomplete blocks, Lawson eastern aspect PSPs are made up of 9 while the northern aspect PSP is made up of 12 incomplete blocks. An incomplete block at Craven is 108m^2 while at Lawson, they are 129.6m^2 therefore the size of a PSP at Craven and the eastern aspect PSPs at Lawson are 0.108 hectares and 0.117 hectares respectively. The northern aspect PSP is the largest and is 0.156 hectares

As stated earlier, the Lawson site has some obvious within-site spatial patterns (slope and aspect) whereas Craven likely has more ambiguous within-site spatial patterns. To ensure nuisance factors were did not interfere with the family variation of, the planting arrangement of *E. bosistoana* progeny at both sites was randomized into the incomplete blocks. Furthermore, the control species acted as a fixed effect in the statistical models to allow for better control of site variation.

As you will be able to see from the maps on the following page (figure 3 and 4), the 'eastern aspect' PSPs are numbered 'PSP 1', 'PSP 2', and 'PSP 3' whilst the 'northern aspect' PSP is numbered 'PSP 4'. Within the results section, they will be referred to as Lawson East and Lawson North respectively. If referring to the a specific PSP at Craven, I will state so, otherwise Craven will be referring to all of the PSPs within that trial.



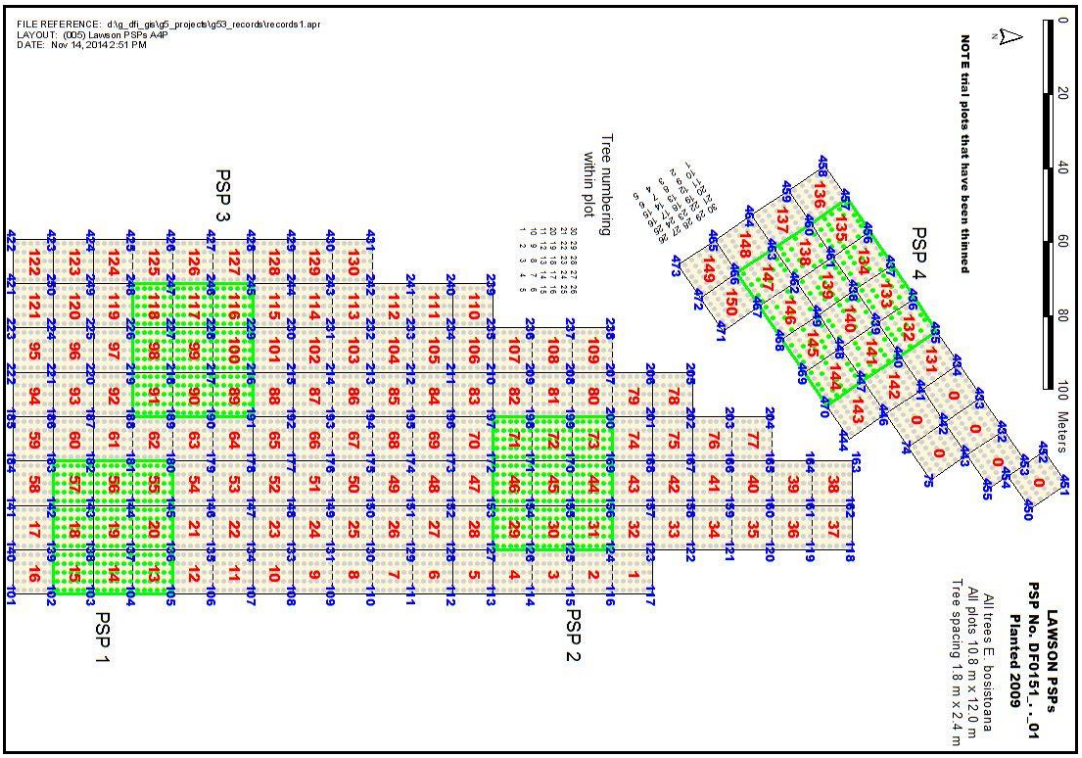


Figure 3 and 4: Craven (top) and Lawson (bottom) site maps, Wairau River is north of Craven trial and ridge of hill at Lawson runs diagonally through trial dividing eastern and northen PSPs.

7.4 Data analysis

To begin the analysis, exploratory data analysis methods were carried out to investigate the overarching relationship between dbh and tree height from all observations and to find if there was evidence of any clear differences between sites. Simple plots and linear models of height against dbh were developed to facilitate this analysis. Site, and PSP averages, minimums and maximums of growth traits were calculated.

The next step was to graph boxplots of dbh and tree height to compare sites against one another as well as comparing individual PSPs against one another. ANOVA calculations of site differences were investigated to analysis if sites were statistically different from one another. I also developed graphs to show the proportional break down of dbh and height at each site. Dbh bins were set up in 10 millimetre increments while height bins were set up in 1 metre increments, which showed total variation of each site.

As I have already mentioned, there was spatial heterogeneity at Lawson whereas Craven could be more safely assumed to be relatively homogenous across the PSPs within the trial. To help account for the heterogeneity at Lawson, the data was grouped into; Lawson North, and Lawson East. The next task was to estimate the degree of genetic control on the growth traits of *E. bosistoana* at the two sites.

A complex mixed-effect linear model was required to assess family variation as accurately as possible. It is the most appropriate model as it separates the random effects from family genotypes, the random effects of incomplete blocks as well as the fixed effects from the control species and the overall mean. The remaining variation from environmental and measurement error is also included. This can be written as;

$$Y_{gh}^{(\text{observations})} = \mu^{(\text{overall mean})} + \beta_g^{(\text{block effect})} + \rho_h^{(\text{family effect})} + \varepsilon^{(\text{residual})} \qquad \text{(Eq.7.4.1)}$$

Where;

- μ is the vector of fixed effects (mean and control effects).
- $\boldsymbol{\beta_g}$ is the random effect of each incomplete block 'g'.
- $\boldsymbol{\rho_h}$ is the random effect of 'h' family (genotype classification) which is also the breeding value of 'h' family for the dataset being evaluated (i.e. breeding

value could be for a family within a PSP or within a site or overall performance across both sites.

- and **ε** is the residual error which includes the non-additive genetic effects, environmental effects, and measurement error.

Utilising the software program 'R' and additional packages 'ggplot2' and 'lme4', I was able to run models for growth traits at Lawson and Craven individually, which returned the variances for all aspects of the model. The variances could then be used to estimate heritability at each site using the formula below (equation 6.4.2). Correlation was calculated using a built-in function within 'R'. Firstly, correlations of growth traits were made between sites and then correlations were made between Craven, East- and North-Lawson.

$$h^2 = \frac{4\sigma_f^2}{\sigma_f^2+\sigma_b^2+\sigma_e^2} \quad \text{(Eq. 7.4.2)}$$

Additional to estimating heritability of growth traits, I investigated family performance at both sites. I exported the random effects of each family that were produced by the mixed-effects linear model, which can be thought of as a family's breeding value. I graphed breeding values of growth traits separately and only graphed families that were present at both sites. I wanted to see how the performance of a family at Craven compared with the same family at Lawson.

By obtaining the breeding values, I was able to rank families for their performance at individual sites (site-specific performers) and rank families based on their breeding values summed from both sites and their breeding values summed from all three distinct groups (Craven, Lawson East and North). Due to the limited number of observations with the PSPs at Craven, it was not possible to calculate breeding values from individual PSPs however; I have calculated individual PSP breeding values for Lawson and have therefore, ranked the breeding values from these PSPs to investigate any consistent performers within one site.

8.0 Results

8.1 Exploratory data analysis

Initial plotting of the data also shows that there is a small level of heteroscedasticity (figure 5). The Craven trial has a much larger spread of measurements in comparison to the Lawson trial. There is a substantial amount of overlap between the two sites; the only obvious difference between the sites is that the *E. bosistoana* trees at the Craven trial have grown quicker than at the Lawson trial but their trajectory seems to be following the same growth path. The Linear model calculated by 'R' gave an intercept of 2.4 metres and a slope of 0.053 (i.e. for every one millimetre increase in dbh, the model predicts a tree will on average be 0.053 metres or 53 millimetres taller).

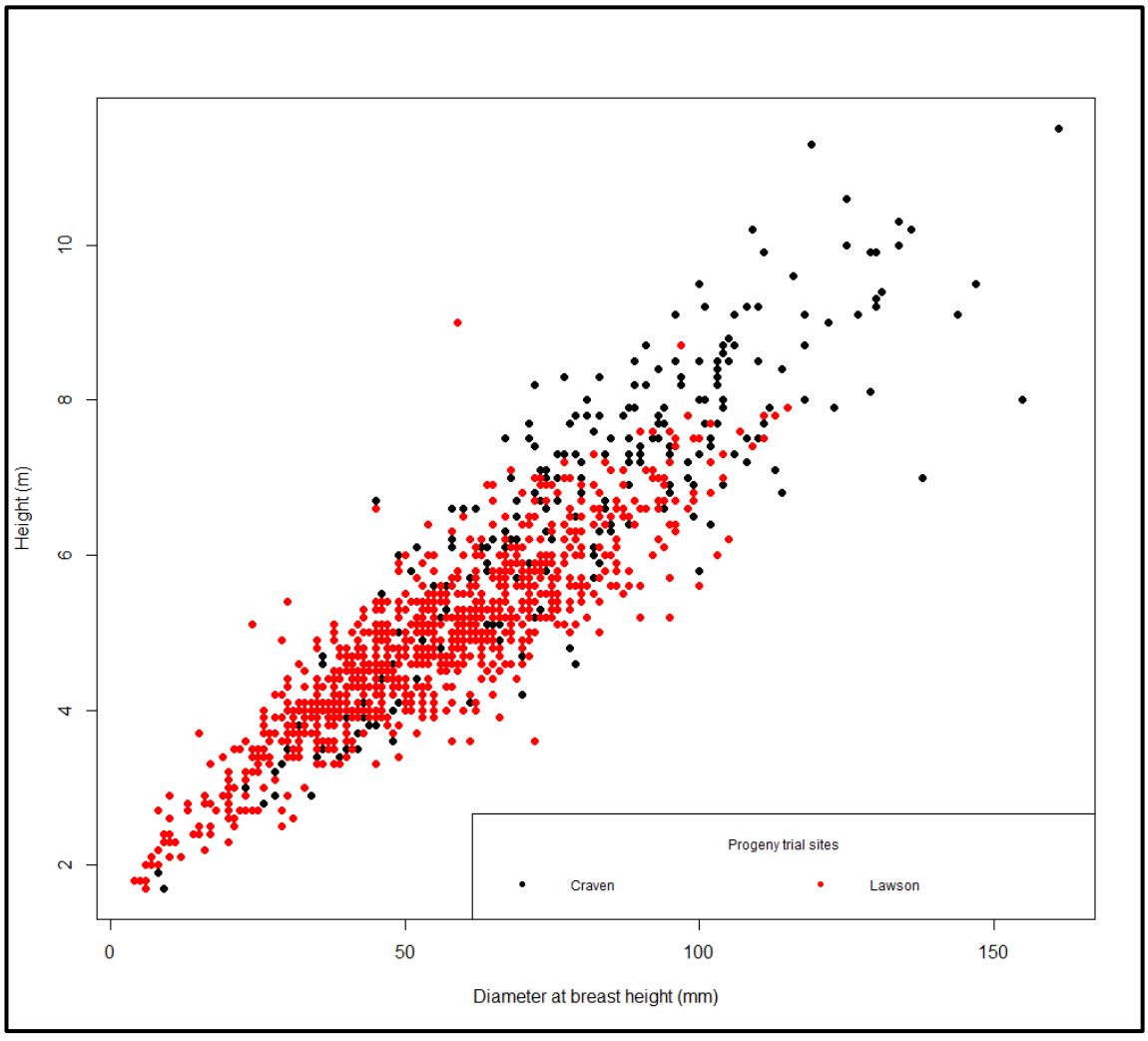


Figure 5: Scatterplot of all observations of diameter and tree height measurements from, Craven are the black dots and Lawson the red.

8.2 Distribution of measurements

The distribution range of dbh and tree height measurements for Craven is much wider than it is for Lawson measurements (figure 6 and 7). Comparing the normality site distributions, Craven represents a normal distribution more closely for both dbh and height. The p-value from shapiro-wilk tests for Craven dbh and height distributions was 0.19 and 0.23 respectively. For Lawson dbh and height distributions, the p-value was 0.008 and 0.001 respectively.

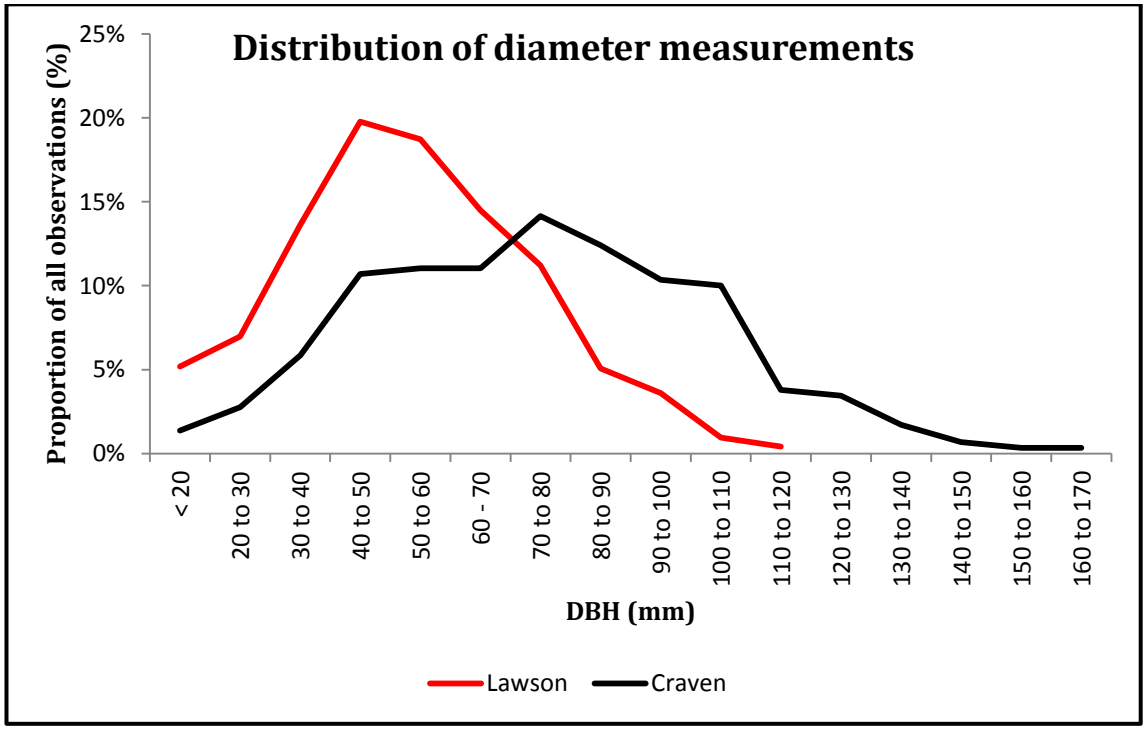


Figure 6: Line graph distribution of binned data proportional to total number of observed measurements for diameter at breast height at both sites.

The highest concentration of tree height measurements at Lawson was found to be between 4 and 5 metres (35%). The dbh measurements were much more evenly spread out across the range of possible values. For Craven, the highest concentration of tree height measurements was found to be between 6 and 7 metres (21%) but this was not extremely dissimilar to concentrations of measurements in other bins (figure 7). Like Lawson, the dbh measurements were much more evenly spread over the entire range. This should be expected due to the scale of the bins used however, both dbh and tree height bins were scaled to

the same degree (10 times the precision of recorded measurements) i.e. dbh measured to nearest millimetre (bins equivalent to nearest centimetre) and tree height measured to nearest 10 centimetres (bins equivalent to nearest metre).

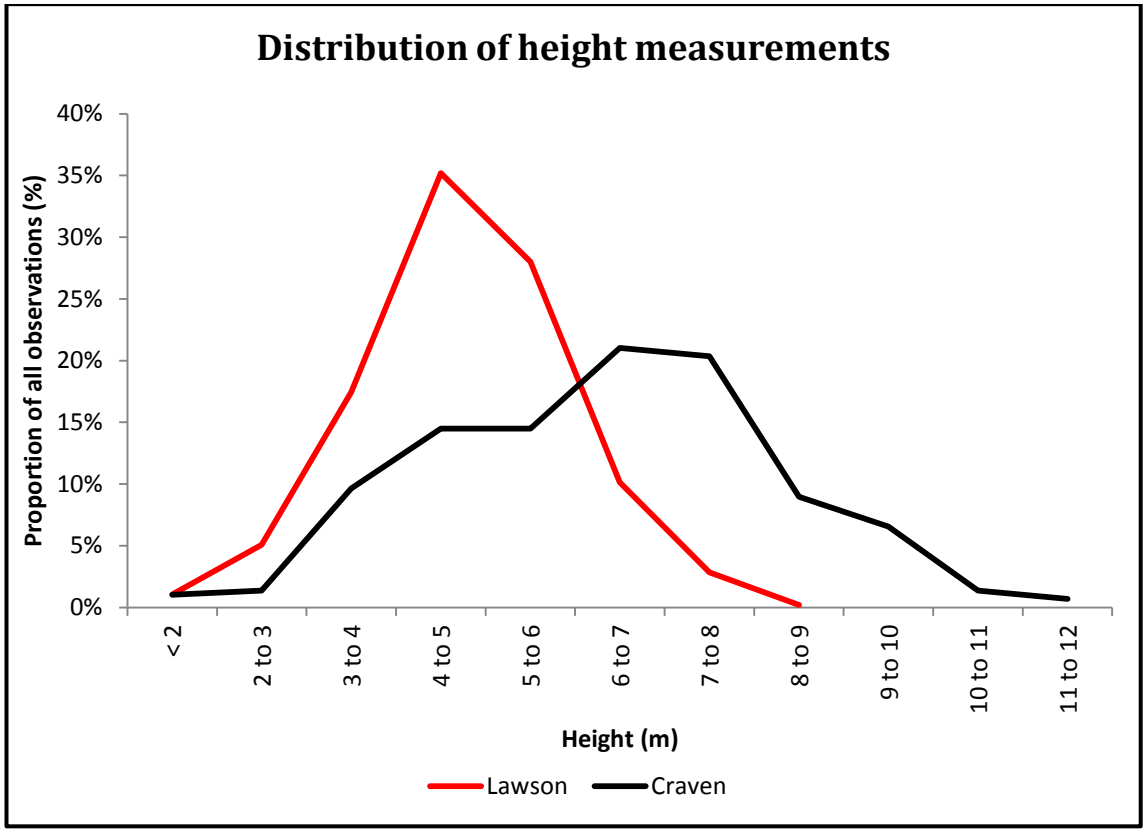


Figure 7: Line graph distribution of binned data proportional to total number of observed measurements for tree height at both sites.

The smallest tree at Craven was six millimetres in diameter and 1.7 metres tall, which was similar to that of the smallest tree at Lawson, it was four millimetre in diameter and 1.7 metres tall. The largest tree at each site on the other hand was quite different; the largest tree at Craven was 46 millimetres larger in diameter (161 millimetres) and 2.5 metres taller (11.5 metres) than the largest at Lawson. Figure 6 illustrates the range of dbh and height measurements observed at both sites; the average dbh and height measurements were; 76 millimetres and 6.4 metres respectively for Craven; while 54 millimetres and 4.8 metres were the calculated dbh and height averages at Lawson (figure 8).

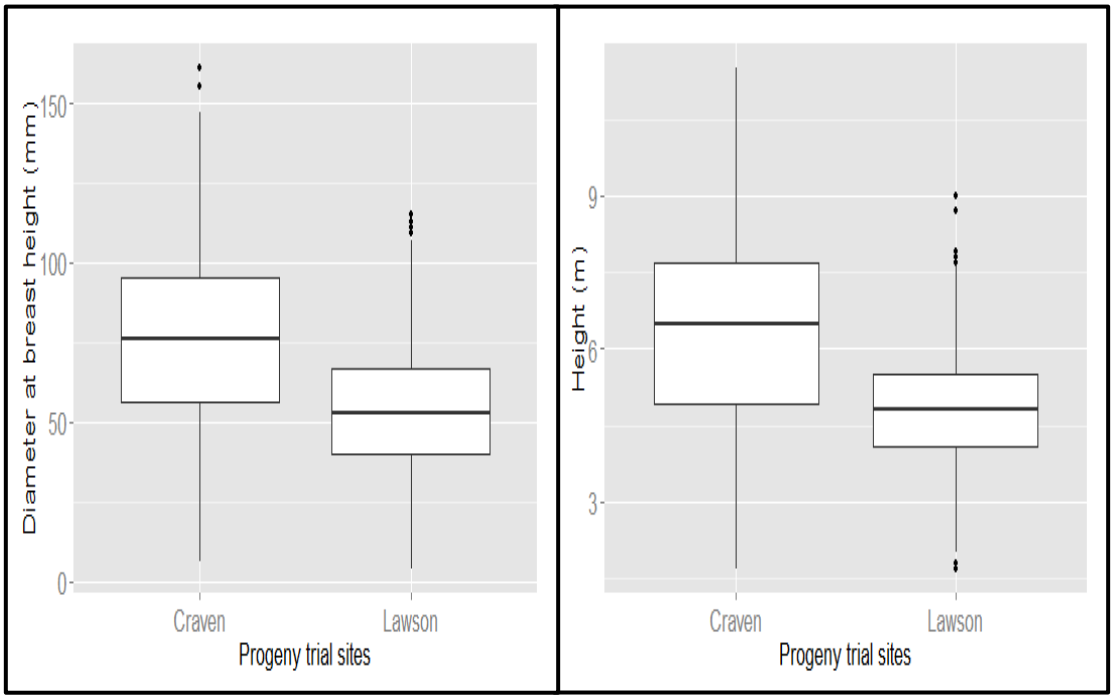


Figure 8: Diameter at breast height and tree height box plots comparing results from Craven and Lawson against one another

Returned p-values from an analysis of variance (ANOVA) test for site differences between dbh measurements and height measurements found that both were significant (p-value < 0.01). That is to say on average, trees at Craven are taller than trees at Lawson. It should be noted here that this is likely to be a result of the earlier thinning that occurred at Craven compared to Lawson only having the northern aspect thinned at the same time. So to analyse it more accurately, I plotted boxplots of all PSPs against one another (figure 9 and 10).

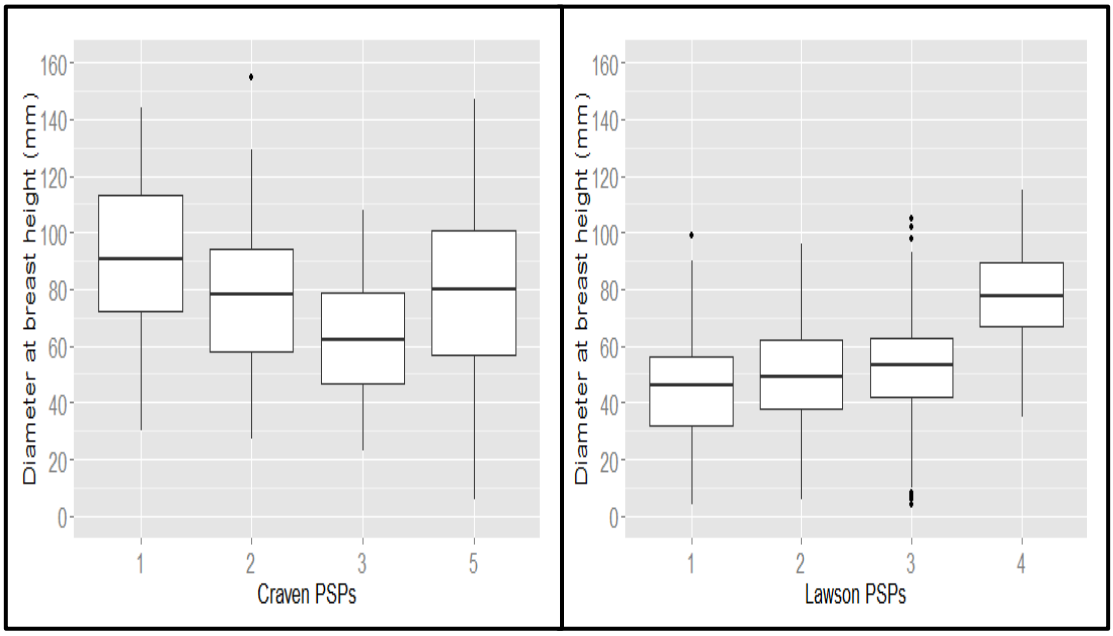


Figure 9: Boxplot of Craven and Lawson diameter at breast height measurements from all PSPs

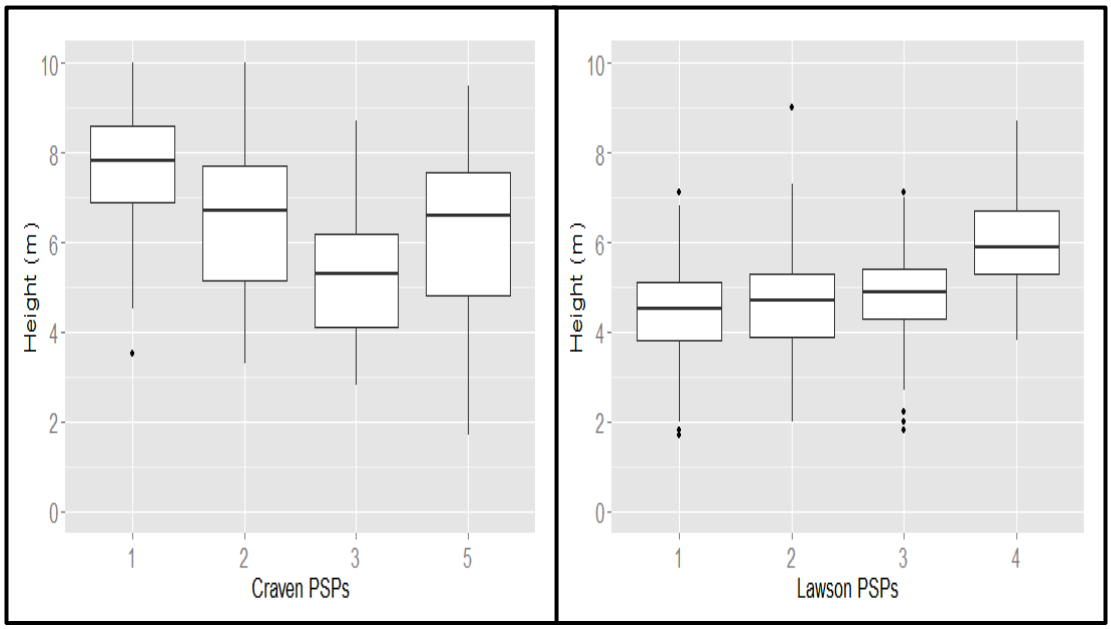


Figure 10: Boxplot of Craven and Lawson tree height measurements from all PSPs

Illustrated by the side by side scatter graphs on the following page (figure 11), there doesn't seem to be a substantial difference between the Craven PSP measurements for dbh or tree height and Lawson North. Table 1, shows the p-values from all ANOVA comparisons that I made. Unsurprisingly, the only comparison that was not significantly different from one another was the dbh measurements from Craven and North Lawson however; the ANOVA produced a p-value < 0.01 for tree height of the same comparison.

Table 1: P-values for ANOVA assessments on diameter at breast height and tree height measurements for multiple scenarios

	DBH	HEIGHT
	ANOVA P-value	ANOVA P-value
Lawson East vs North	< 0.001	< 0.001
Lawson East PSPs	< 0.001	< 0.001
Craven PSPs	< 0.001	< 0.001
North Lawson/Craven PSPs	**0.5**	< 0.01

The dispersion of measurements within Craven and Lawson North show the evidence of the thinning operations. Of interest from figure 11 is the fact that although thinning operations have been carried out in these groups, the range of measurements is still representative of an unthinned group (Lawson East).

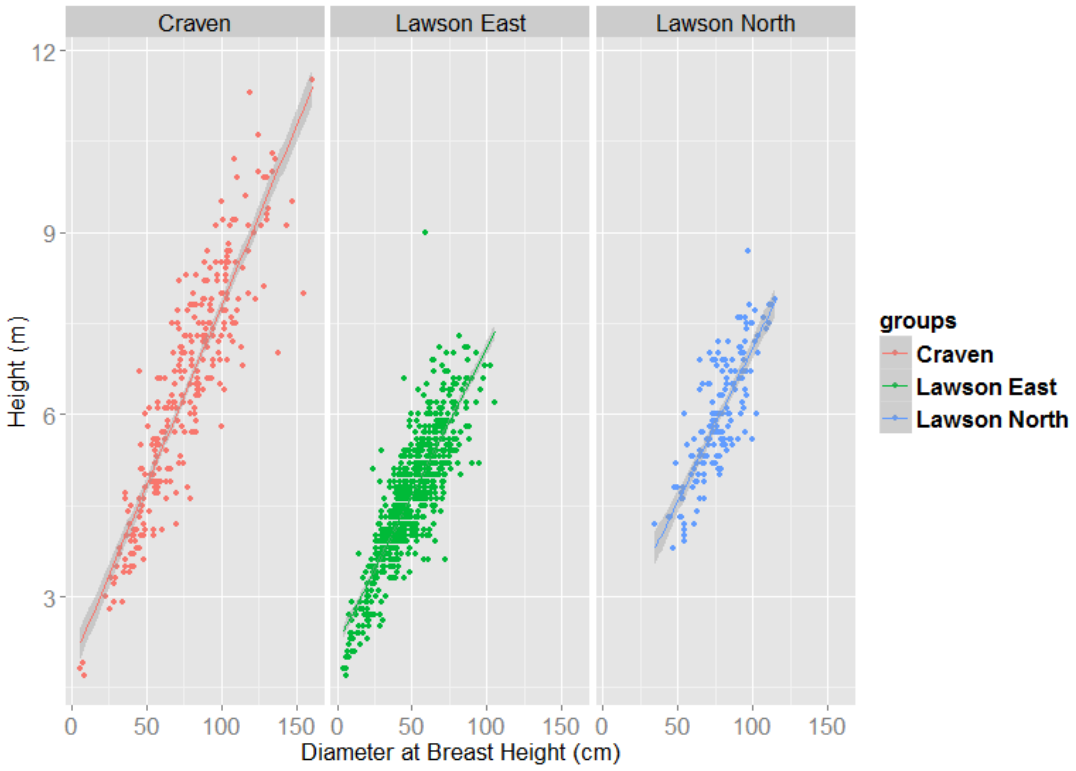


Figure 11: Side by side scatter graphs of height against diameter at breast height from the three distinct groups.

8.3 Diameter at breast height and tree height variance

The mixed-effect linear model provided further evidence that Craven on average has taller trees and trees with larger diameters (table 2). Additionally Craven had a higher family, incomplete block, and residual variance than Lawson which should not be surprising as figure 11 displayed the difference in the distribution of points. I was interested in minimising any environmental noise as much as possible and after separating Lawson East from Lawson North and re-calculating the model variances, most of the estimates for heritability increased as a result (table 2).

The heritabilities for dbh growth trait that I calculated for Craven, Lawson North and Lawson East are 0.18, 0.13, and 0.17 respectively. For tree height the heritabilities calculated are 0.10, 0.09, and 0.17 with respect to the same order as before.

Table 2: Random effects and residual effects from the mixed-effect linear model for site comparisons along with estimation of heritability

		Mean	Random effects		Residual effects	Estimate of Heritability
		μ	σ^2 (family)	σ^2 (incomplete block)	σ^2	h^2
Craven	height (m)	**6.4**	0.148	1.999	1.427	**0.10**
	dbh (mm)	**75**	58	323	425	**0.18**
Lawson	height (m)	**5.0**	0.051	0.599	0.793	**0.09**
	dbh (mm)	**57**	21	202	255	**0.11**
Lawson North	height (m)	**5.9**	0.038	0.538	0.466	**0.09**
	dbh (mm)	**77**	17	88	146	**0.13**
Lawson East	height (m)	**4.6**	0.053	0.143	0.859	**0.17**
	dbh (mm)	**49**	21	21	278	**0.17**

Additional to estimating heritabilities from the variance of random effects and residual, the fixed effects of control (alternative species planted within the trials) showed that at Craven, all controls performed better in terms of their diameter growth but not differently in terms of the tree height growth (table 3). At Lawson however, the only notable diameter difference in control from the *E. bosistoana* plantings was control 997, on average it had a larger diameter whereas the other control plantings all had smaller diameters. In terms of control effects on height at Lawson, the only substantial difference came from control 999, it was on average 0.9 metres smaller than the *E. bosistoana* plantings.

Table 3: Fixed effects of control plantings from the mixed-effect linear model for site comparisons

	Diameter		Height	
	Craven	Lawson	Craven	Lawson
Estimate (site mean)	75.5	57.4	6.4	5
Control – 997 (E. globoidea)	+ 11	+ 13	-0.4	+ 0.2
Control – 998 (E. quadrangulata)	+ 30	- 1	+ 0.3	-0.5
Control – 999 (E. globoidea)	+ 21	- 2	-0.2	-0.2
	Diameter		**Height**	
	Lawson North	Lawson East	Lawson North	Lawson East
Estimate (site mean)	77	49	5.9	4.6
Control – 997 (E. globoidea)	+ 13	+ 9	+0.2	+ 0.2
Control – 998 (E. quadrangulata)	- 1	- 1	-0.2	-0.6
Control – 999 (E. globoidea)	- 8	- 1	-0.9	-0.1

8.4 Breeding values of growth traits

Breeding values were found to be distributed widely for both dbh and tree height. There are examples of families performing similarly at both sites (could mean they perform badly at both or reasonably well at both) and other examples of families that are exceptional (or conversely, have very low breeding values) on one site and perform at the average or above at the other site (figure 12 and 13).

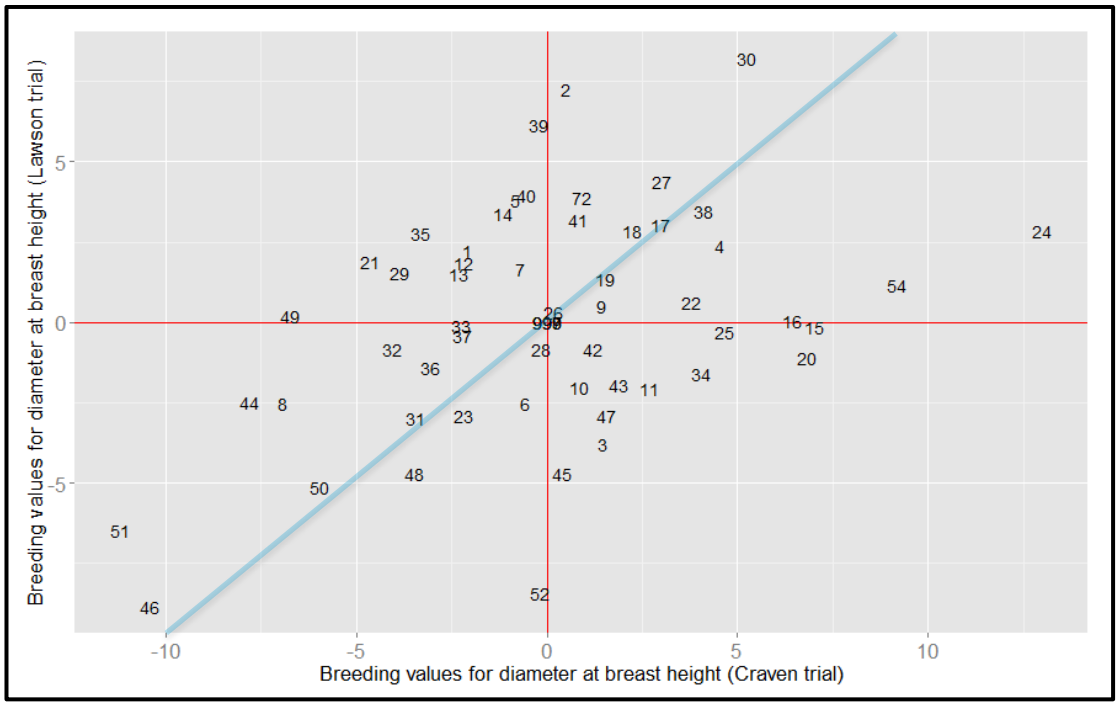


Figure 12: Breeding values for tree height of the families that were recorded at both sites.

The diagonal line on figures 12 and 13 depicts the line where families perform equally well or badly at both sites i.e. the correlation of the individual family breeding values between sites would theoretically equal 1. The top right square of figures 12 and 13 represents families that have breeding values greater than the average family at both sites while the top left and bottom right squares represent families that have breeding values better than the average at one site but worse than the average at the other site.

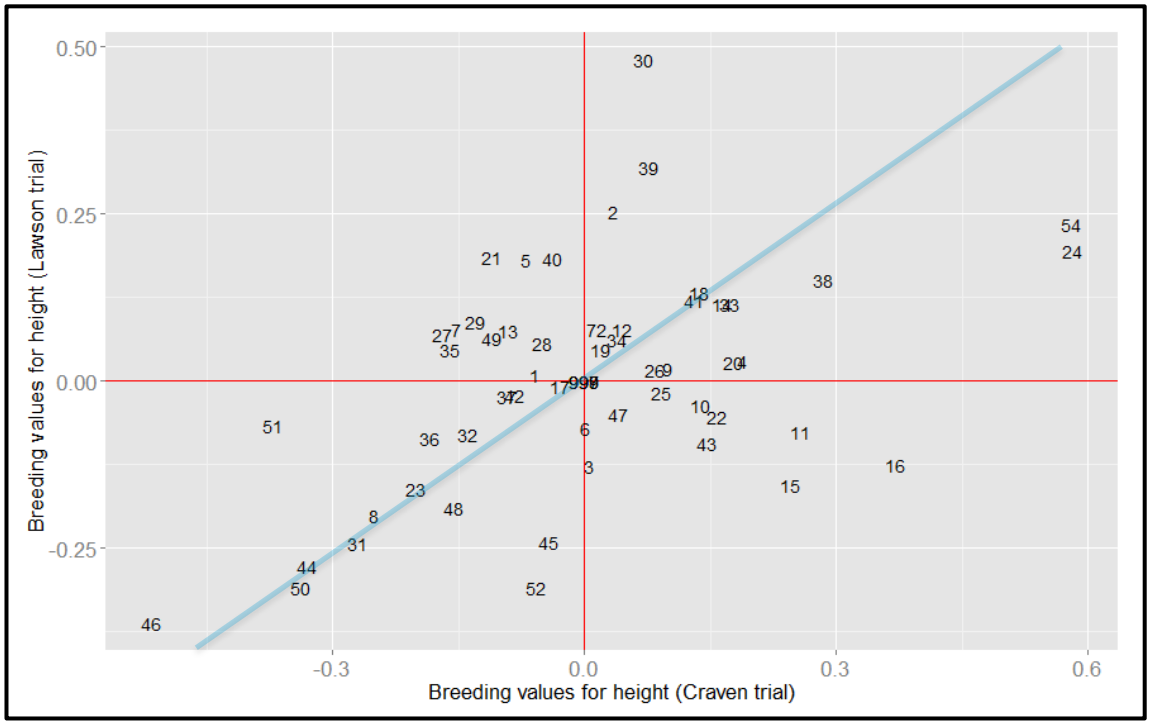


Figure 13: Breeding values for diameter at breast height measurements of the families that were recorded at both sites

The correlation of breeding values between sites for height was found to be 0.47 whilst the correlation of dbh breeding values was found to be slightly less at 0.41. Although I have not produced a graphic of Lawson East and Lawson North breeding values, the height correlations were calculated and are as follows;

- Craven : Lawson North = 0.28
- Craven : Lawson East = 0.44
- Lawson East : Lawson North = 0.27

With regard to the dbh correlations between sites for breeding values, they are;

- Craven : Lawson North = 0.32
- Craven : Lawson East = 0.39
- Lawson East : Lawson North = 0.36

8.5 Family ranking

In the tables below, I have put together on the top 10 families based on the average breeding value they obtained across all three sites and have then displayed the breeding value they obtained at each site. A graphic of this can be found in the appendix. Table 4 is represents the breeding values from dbh measurements whilst table 5 represents the breeding values for tree height. As you can see, there are commonalities between families that appear in the top 10 for dbh and height; 24 tops the rankings for both sites and families 30, 54, 38, 18, and 2 appear in the top rankings for both growth traits.

Table 4: Specific breeding values of the top 10 ranked families with respect to diameter measurements. Ranking is based on the average breeding value across all three sites but the table has the actual breeding values calculated for each site

Family	Lawson North	Craven	Lawson East
24	2.08	12.98	2.33
30	5.75	5.24	5.92
2	2.35	0.49	6.91
54	-1.95	9.17	2.01
38	0.79	4.13	3.47
17	3.10	2.98	2.06
4	1.14	4.55	2.07
18	3.91	2.23	1.42
27	-0.51	3.01	4.66
15	0.02	7.02	-0.12

No families from codes 55 – 73 appeared in the top rankings, which is material derived from CSIRO. Forest Seeds Australia and Forests New South Wales have five families representing their seed each with regard to the dbh breeding value rankings. In the tree height breeding value rankings, Forest Seeds Australia has three families while Forests New South Wales comprise the rest of the top 10 ranked families representing their seed.

Table 5: Specific breeding values of the top 10 ranked families with respect to tree height measurements. Ranking is based on the average breeding value across all three sites but the table has the actual breeding values calculated for each site

Family	Lawson North	Craven	Lawson East
24	0.084	0.581	0.163
54	-0.016	0.580	0.258
30	0.262	0.070	0.370
38	0.067	0.284	0.125
39	0.045	0.076	0.309
33	0.108	0.173	0.062
18	0.104	0.137	0.083
14	0.059	0.164	0.096
2	0.027	0.034	0.258
41	0.026	0.131	0.112

9.0 Discussion

Breeding programmes are more successful when there is genetic variation of a desired trait and that same trait can be selected for effectively (that it is heritable). This report has provided results that can be used as evidence to support the goals of breeding programmes.

Given that these trials are the first of its kind for this particular species, it really is in a similar position to where *Pinus radiata* was when breeding programmes first began for it here in New Zealand. There are benefits and drawbacks to starting this venture as early as it has. Firstly, New Zealand has 50 years of experience in breeding trees under its belt. This provides NZDFI with an array of information and data to consult with enabling better educated decisions to be made around site choice, what to breed for, or which species (Wilcox, 1980) (Burdon, 2008). On the other hand, starting an entire breeding programme entails years of work as seed has to be propagated in nursery conditions for at least six months (NZDFI, 2015). It is not until the progeny begin flowering that crosses can be made from the selected species, luckily many of the trees at both sites have already began flowering. The only related issue to that is the timing of inflorescence, two desirable plus trees need to have their inflorescence present at the same time period, which can be unlikely at times.

The additive genetic variation observed within this study at both sites was relatively small which led to low heritabilities being calculated. The fact that the progeny are open pollinated half-sibs has negatively influenced the potential heritability for the growth traits to be slightly higher. Although past research with other species has shown that the heritability of growth traits can range from 0.1 to in excess of 0.5, two of heritabilities of dbh growth trait were very similar to the average calculated from past research of other species while Lawson North was substantially lower.

Comparing the findings from Luis et al. (2011) of height heritability at an early age (16 months) of the same trial sites (Craven = 0.13, Lawson = 0.1), I found that the heritabilities I had calculated (Craven = 0.10, Lawson North = 0.09, Lawson East = 0.17) were relatively consistent. It appears that *E. bosistoana* may be a species that has low to medium heritability for growth traits, however, this report and the study by Luis et al (2011) are the only trials that have been assessed for genetic

parameters of growth thus, further studies completed outside of Marlborough or at least at different sites are required to confirm this conclusion.

At looking for an explanation of the large residual variance within-sites, one could possibly to infer that it could be any number of factors at each site, for instance; slope, soil gradients and changes within-site, competition from trees within the trial or neighbouring trees, drought, frost days etc. The exception would be that if *E. bosistoana* were planted on a site with soils consisting of loams over limestone as the Forestry and Timber Bureau (1962) stated that this was the trees preference and Craven resembles a loam soil much closer that Lawson does.

It was possible to link the thinning operations to actual observed differences in the progeny trials however, which had more of an effect on diameter growth than it did on trees height. This idea can be reinforced with experiments utilising the Nelder Wheel (Waghorn, Mason, & Watt, 2007). Wider tree spacings increase the amount of light that is filtered through the forest therefore, the trees are not competing with their neighbours for photosynthetic energy by putting more energy into height but rather directing their flow to becoming stable.

The breeding values show that there is potential for selection of this species where families could be crossed with one another to be bred for specific sites or selections could be made to encourage trees to perform homogeneously across multiple sites. The lower to medium level of correlation of the breeding values indicates that there are more trees that perform superiorly at one site over the other. It turned out that more often than not that top ranked families from Lawson North, Lawson East, or Craven could be found in the top ranked families of another site. So, although the correlation was not high between sites, their were at least some of the same families consistently ranking the top of their site based on breeding values.

10.0 Conclusion

Tree breeding is an expensive, capital intensive, time hungry investment which requires a lot of hands on, groundwork to ensure you are capturing all the necessary data that you require for genetic analysis. These results have shown that larger numbers of families and trials may be required to increase the frequency of plus trees within progeny trials as only a handful of standout families showed real promise.

Given that the trees have begun to flower, studies can be carried out on the viability and timing of control pollinating plus trees. From there selections of plus trees can be made and the breeding programme can move on to applying more research effort to understanding genetic control of wood quality traits. Presently, efficient tools are lacking that can carry out a sufficient level of observations for the breeders to analyse.

This report presents the second genetic assessment at age 5 that has been carried out on these trials (the first was completed at 16 months) and is intended to add to the current literature on this topic and species. The growth traits both had large amounts of variation within- and between-family at Craven and Lawson which is one requirement of a successful breeding programme however, there was a relatively low level of heritability for these traits (h^2 ranged from 0.9 – 0.18).

The low level of correlation between sites indicated that there is a moderate amount of genotype by environment interaction apparent for both traits (dbh = 0.32 to 0.39, height 0.27 to 0.44) which contradicts findings by Luis et al. (2011) but there may be some opportunities to deploy progeny with particular characteristics.

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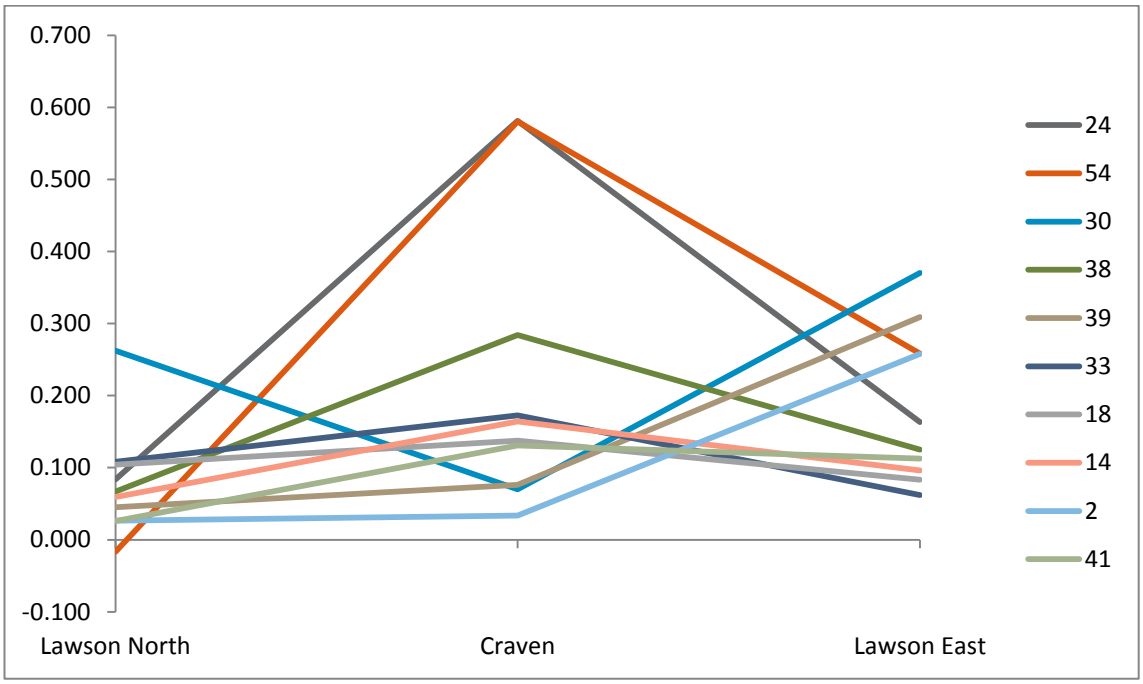
Appendix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Lawson North
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Lawson East
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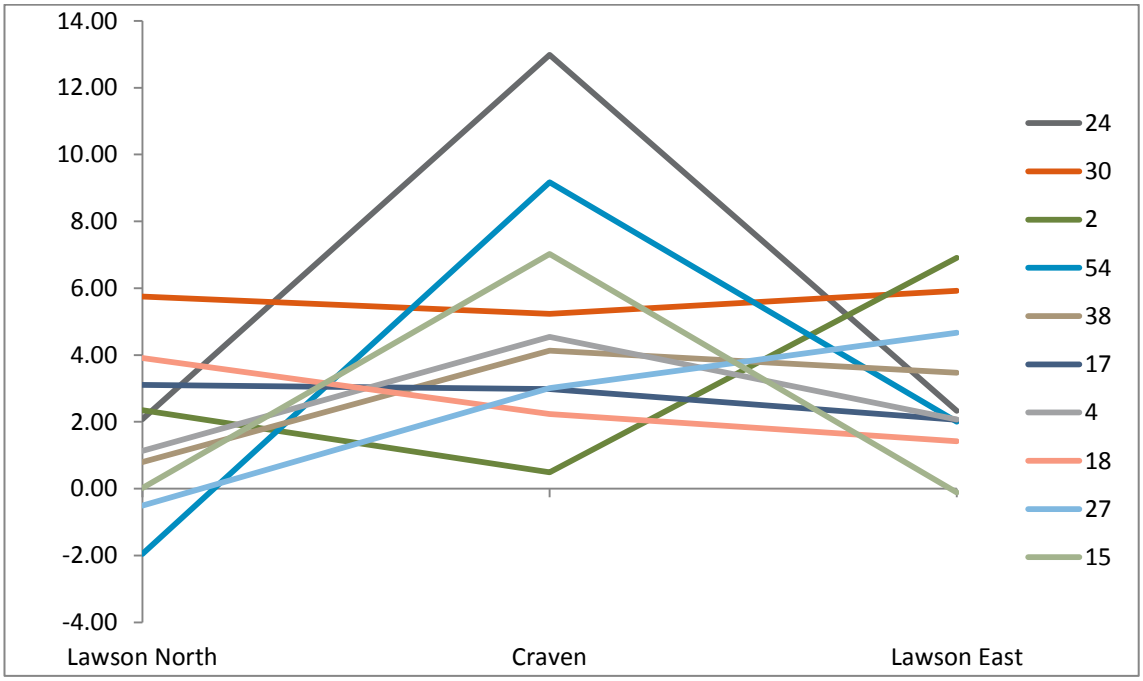
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Breeding values			
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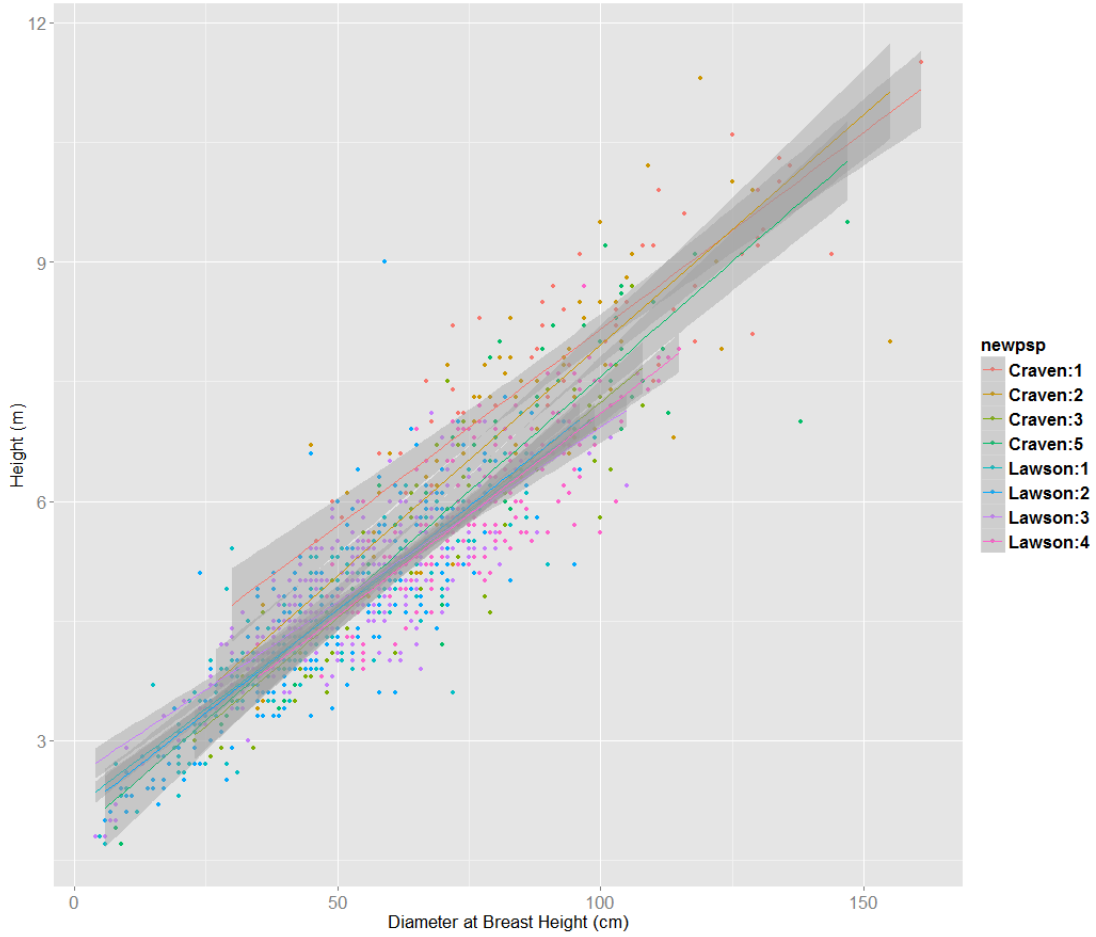

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.222005	0.2632687	12.238	< 2e-16
dbh	0.0493743	0.0026919	18.342	< 2e-16
Craven:2	-1.0452012	0.3380183	-3.092	0.002032
Craven:3	-1.3944463	0.3363493	-4.146	3.62E-05
Craven:5	-1.4103473	0.3330065	-4.235	2.46E-05
Lawson:1	-1.0637138	0.2824516	-3.766	0.000174
Lawson:2	-1.1673661	0.284587	-4.102	4.37E-05
Lawson:3	-0.6793127	0.2890817	-2.35	0.018937
Lawson:4	-1.2040881	0.3544835	-3.397	0.000704
dbh:Craven:2	0.0084801	0.003751	2.261	0.02395
dbh:Craven:3	0.0047885	0.0041201	1.162	0.245374
dbh:Craven:5	0.0081734	0.0036696	2.227	0.026107
dbh:Lawson:1	0.0001405	0.0034188	0.041	0.967228
dbh:Lawson:2	0.0023776	0.0033886	0.702	0.483032
dbh:Lawson:3	-0.0055087	0.0034544	-1.595	0.111036
dbh:Lawson:4	0.0014391	0.0040244	0.358	0.720714